410 Rec'd PCT/PTO 31 MAY 2000

FORM PTO-1390 (REV 11-98)	U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 2801-18
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S APPLICATION NO. (If known, see 37 C.F.R 1.5) 09/555473 (To be assigned)
INTERNATIONAL APPLICATION NO. PCT/IT98/00364	INTERNATIONAL FILING DATE 16 December 1998	PRIORITY DATE CLAIMED 19 December 1997

TITLE OF INVENTION

PHARMACEUTICAL COMPOSITIONS CONTAINING THE LONG PENTRAXIN PTX3

APPLICANT(S) FOR DO/EO/US

BOTTAZZI et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. To 16. Below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information. **International Search Report/ PTO-1449**

431058

U.S. APPLICATION NO (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/555473 (To be assigned)	PCT/IT98/00364	2801-18

17. ☒ The following fees are submitted:

	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5):	
-- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $970.00	
-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $840.00	
-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2) paid to USPTO $690.00	
-- International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $670.00	
-- International preliminary examination fee paid to USPTO (37 C.F.R. 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00	

ENTER APPROPRIATE BASIC FEE AMOUNT =							$	840.00
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).							$	0.00
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE				
Total Claims	12	-20 =	0	X	$18.00		$	0.00
Independent Claims	4	-3 =	1	X	$78.00			78.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)				$260.00			$	0.00
TOTAL OF ABOVE CALCULATIONS =							$	**918.00**
Reduction by ½ for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 C.F.R. 1.9, 1.27, 1.28).								0.00
SUBTOTAL =							$	**918.00**
Processing fee of $130.00, for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)). +								0.00
TOTAL NATIONAL FEE =							$	**918.00**
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). **$40.00** per property +							$	40.00
Fee for Petition to Revive Unintentionally Abandoned Application ($1210.00 – Small Entity = $605.00)							$	0.00
TOTAL FEES ENCLOSED =							$	**958.00**
							Amount to be: refunded	$
							Charged	$

a. ☒ A check in the amount of $958.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 14-1140 in the amount of $_____ to cover the above fees. A duplicate copy of this form is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed.
d. ☐ The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Arthur R. Crawford
NAME

25,327 **May 31, 2000**
REGISTRATION NUMBER Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

BOTTAZZI et al.

Atty. Ref.: 2801-18

Serial No. (To be assigned)

Group:

Filed: May 31, 2000

Examiner:

For: PHARMACEUTICAL COMPOSITIONS
CONTAINING THE LONG PENTRAXIN PTX3

* * * * * * * * * * *

May 31, 2000

Assistant Commissioner for Patents
Washington, DC 20231
Sir:

PRELIMINARY AMENDMENT

In order to place the above-identified application in better condition for examination, please amend the application as follows:

IN THE CLAIMS

Claim 4, line 1, change "claims 1-3" to --claim 1--.

REMARKS

The above amendments are made to place the claims in a more traditional format.

Respectfully submitted,

NIXON & VANDERHYE P.C.

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Pharmaceutical compositions containing the long pentraxin PTX3

The present invention relates to pharmaceutical compositions containing the long pentraxin PTX3 (PTX3) or one of its functional derivatives. In particular, the invention relates to the aforesaid compositions for the therapy of infectious and inflammatory diseases or tumours.

The invention also relates to expression vectors containing the complete cDNA sequence coding for PTX3 or one of its functional derivatives, recombinant host cells transfected with such expression vectors and a method for producing PTX3 or one of its functional derivatives. Further, the invention relates to gene therapy methods for the treatment of tumours, based on the use of the aforesaid expression vectors.

To date, we have yet to fully understand the biological function of PTX3, a protein which is expressed in various types of cells, most notably in mononuclear phagocytes and endothelial cells, after exposure to the inflammatory cytokines Interleukin 1beta (IL-1beta) and Tumour Necrosis Factor alpha (TNF-alpha).

To date, there has also been no description of any therapeutic use of PTX3 or of its functional derivatives.

PTX3 consists of two structural domains, an N-terminal unrelated to any known molecule, and a C-terminal similar to the short pentraxins such as C-reactive protein (CRP). A substantial degree of similarity has been found between human PTX3 (hPTX3) and animal PTX3s.

The PTX3 gene is located on chromosome 3 of the mouse in a region similar to the human 3q region (q24-28), in agreement with the documented location of hPTX3 in the 3q 25 region. Furthermore, mouse PTX3 (mPTX3) (Introna M., Vidal Alles V., Castellano M., Picardi G., De Gioia L., Bottazzi B., Peri G., Breviario F., Salmona M., De Gregorio L., Dragani T.A., Srinivasan N., Blundell T.L., Hamilton T.A. and Mantovani A.: Cloning of mouse PTX3, a new member of the pentraxin gene family expressed at extrahepatic sites. Blood 87 (1996) 1862-1872) is very similar to hPTX3 in terms of organisation, location and sequence (Breviario F., d'Aniello E.M., Golay J., Peri G., Bottazzi B., Bairoch A., Saccone S., Marzella R., Predazzi V., Rocchi M., Della Valle G., Dejana E., Mantovani A., Introna M.: Interleukin-1-inducible genes in endothelial cells. Cloning of a new gene related to C-reactive protein and serum amyloid P component. J. Biol. Chem. 267:22190, 1992).

In particular, the degree of identity between the sequences is 82% between the human and mouse gene and reaches 90% if conservative substitutions are considered.

The high degree of similarity between the hPTX3 and mPTX3 sequences is a sign of the high degree of conservation of pentraxin during evolution (Pepys M.B., Baltz M.L.: Acute phase proteins with special reference to C-reactive protein and related proteins (pentraxins) and serum amyloid A protein. Adv. Immunol. 34:141, 1983).

CRP is a marker for immuno-inflammatory and infectious disease. After a trauma, a lesion or infection of a tissue triggers off, in the affected subject, a complex series of reactions aimed at preventing extension of the damage, at destroying the infecting organism and at activating the repair process in order to restore normal function. This process constitutes the so-called acute-phase response, and the main marker currently used for the acute-phase response is CRP. In normal human serum, in fact, it is present in concentrations of less than 10 μg/ml, but can increase more than 1,000-fold in response to a trauma or inflammation (Koj A.: "Acute phase reactants" in "Structure and Function of Plasma Proteins". Allison A., ed. Plenum Press, New York, 1974, pp. 73-131).

Previous therapeutic uses of CRP are already known. For instance, US Patent 4,857,314 dated 15.08.1989 discloses the use of CRP in combination with TNF for the treatment of tumours.

International patent application PCT/US94/02181 dated 24.02.1994 discloses mutants of CRP which are useful for the preparation of diagnostic kits for determining immunocomplexes in

biological fluids and for the treatment of viral and microbial diseases, tumours and endotoxic shock.

International patent application PCT/US94/09729 dated 26.08.1994 also discloses mutants of CRP which are useful for the preparation of diagnostic kits and for the treatment of viral and microbial diseases and tumours.

The ability of PTX3 to recognise and bind specifically to ligands which are also recognised by short pentraxins has been evaluated *in vitro* using purified recombinant PTX3. Short pentraxins such as CRP and SAP (serum amyloid P component) are characterised by their ability to recognise and bind in a calcium-dependent manner to a broad spectrum of ligands, including phosphocholine, phosphoethanolamine, many sugars, the best characterised of which is an agarose derivative rich in pyruvate [methyl 4-6-O-(1-carboxyethylidene)-beta-D-galacto-pyranoside] or MOβDG, complement fragments and proteins of the extracellular matrix, particularly fibronectin and type IV collagen. Unlike the short pentraxins, PTX3 is unable to bind either calcium (assessed by Inductive Coupled Plasma/Atomic Emission Spectroscopy) or phosphocholine, phosphoethanolamine or MOβDG. Moreover, PTX3 is unable to bind extracellular matrix proteins such as fibronectin or type IV collagen. On the other hand, PTX3 is capable of binding the C1q complement fragment which is also recognised by the short pentraxins (Table 1). It

should be stressed, however, that, whereas CRP and SAP have to be cross-linked to bind C1q, PTX3 is capable of recognising and binding this complement fragment in the naturally occurring form.

Surprisingly, it has now been found that the long pentraxin PTX3 or its functional derivatives are useful therapeutic agents, particularly for the therapy of infectious and inflammatory diseases or tumours.

What is meant by "long pentraxin PTX3" is any long pentraxin PTX3, i.e. regardless of its natural (human or animal) or synthetic origin. Human long pentraxin PTX3 (see sequence 1 and Fig. 5) is the preferred form.

A convenient method of producing substantial amounts of long pentraxin PTX3 or one of its functional derivatives consists in preparing expression vectors (e.g. plasmids) containing the complete cDNA sequence coding for PTX3 or one of its functional derivatives and in using these to transfer eukaryotic cells in culture (e.g. Chinese hamster ovary cells, CHO). After cloning the recombinant host cells thus transfected, the cell clone capable of producing the highest levels of PTX3 is selected.

According to the present invention, the above-mentioned expression vectors containing the cDNA sequence coding for long pentraxin PTX3 are also utilised in gene therapy methods for the treatment of tumour conditions.

A first gene therapy method consists in:

a) taking samples of cells from a patient suffering from a tumour;

b) transfecting these cells with an expression vector containing the complete cDNA sequence coding for long pentraxin PTX3 or one of its functional derivatives; and

c) inoculating the tumour patient with these transfected cells.

A second gene therapy method for the treatment of tumours consists in:

a) preparing an expression vector of viral origin (such as an adenovirus or retrovirus) containing the complete cDNA sequence coding for long pentraxin PTX3 or one of its functional derivatives; and

b) injecting the tumour affected patient with the expression vector thus obtained.

Though the mechanism of action of PTX3 or its functional derivatives has yet to be definitively clarified, their anticancer activity in any event is not attributable to a direct cytolytic or cytostatic effect on the tumour cells, but rather to mechanisms mediated by the host and related to the leukocyte recruitment ability exerted by these compounds, as will be described below.

There now follows a description of the experimental procedures and results are reported demonstrating the unexpected activity of the compounds according to the invention described herein.

Production of recombinant PTX3: a fragment containing the complete cDNA sequence of human PTX3 (sequence 2 and Fig. 6) was subcloned in the Bam H1 site of the expression vector pSG5 (Fig. 1) (Stratagene, La Jolla, CA, USA) and transfected in CHO cells using the precipitated calcium procedure. A clone selected in G418, capable of producing large amounts of PTX3, was used as a source from which the protein was purified by chromatography by means of ion exchange and gel filtration.

Binding of PTX3 to C1q: the binding of PTX3 to C1q was assessed in an ELISA system. A 96-well plate was covered with 250-500 ng of C1q per well (one night at 4°C) and then washed with PBS with Ca^{++} and Mg^{++} containing 0.05% Tween 20 (PBS). The wells were then blocked with 5% milk in PBS (2 h at room temperature) and subsequently incubated with variable concentrations of PTX3 (30 min at 37°C). After a further series of washings, the plate was incubated with a rat monoclonal antibody to PTX3 (1 h at room temperature) and then with the second antibody, a peroxidase-conjugated rat anti-IgG antibody (1 h at room temperature). After washing, chromogen was added and absorbance was read at 405 nm using an automatic plate

reader. In a number of experiments, the wells were covered with PTX3 and C1q binding was evaluated using an anti-C1q antibody.

Biotinylated protein was used to determine the C1q binding affinity. PTX3 was biotinylated according to standard procedures using an activated biotin modified by the addition of a "spacer arm". (SPA – Società Prodotti Antibiotici).

Figures 2(A) and 2(B) give the C1q binding and binding affinity results. These results show the very substantial degree of C1q binding and binding affinity of PTX3.

Leukocyte recruitment: the leukocyte recruitment induced by PTX3 was studied *in vivo* in the subcutaneous pocket system. The subcutaneous pocket was induced in the experimental animal by means of two subcutaneous injections of 5 mL of air into the animal's back with an intervening interval of three days. On day 6, 1 μg of PTX3 in 0.5% carboxymethylcellulose was administered into the pocket. After 4 h, the animals were anaesthetised and the pocket was washed with 1 mL of saline solution. The washing liquid was recovered and was submitted to a total count and a differential count of the cells present.

The results obtained are reported in Figure 3 and show the substantial leukocyte recruitment capacity of PTX3 in normal animals, whereas Figure 4 shows the results obtained in genetically modified

animals, without C1q, in which the leukocyte recruitment is significantly lower.

Anticancer activity: a line of murine mastocytoma P815 was co-transfected by electroporation with the expression vector pSG5 containing the cDNA of human PTX3 or its antisense and the vector pSV2 which endows the transfected cells with neomycin resistance. After selection with neomycin 0.5 mg/mL, the cells were cloned by limit dilution.

To assess the production of PTX3, $2.5x10^5$ cells were cultivated in 200 μL of RPMI + 3% FCS for 24 h and the supernatant was tested by ELISA. The clones obtained produced protein levels ranging from 1 to 35 ng/mL, while the clones containing the antisense produced no measurable levels of PTX3. The clones considered showed the same growth rate *in vivo*.

Male DBA/2N CrlBR mice aged 8-10 weeks were subcutaneously injected with $1x10^5$ cells of P815 PTX3-producing clones or with clones containing the antisense gene. The mice were monitored 3 times daily for occurrence of tumours and once daily for survival.

The results obtained are reported in Table 2 and show the efficacy of PTX3, in this experimental model of gene therapy, in bringing about healing of the animals and complete rejection of the tumour after the take of the inoculated tumour cells.

These results are statistically significant with $p<0.01$ (Fisher test) both as compared to controls and to the group treated with the antisense.

In the light of these results it is clear that the anticancer activity reported above correlates closely with the leukocyte recruitment which occurs in the mouse as a result of recognition of the C1q by PTX3. In genetically modified mice, no such leukocyte recruitment occurs. The leukocyte recruitment capacity, on the basis of the anticancer activity of the compounds according to the invention, indicates that these compounds may also have a useful application in the treatment of diseases caused by pathogens such as bacteria, fungi, protozoa or viruses.

TABLE 1 PENTRAXIN BINDING ABILITY TO VARIOUS LIGANDS

	CRP	SAP	PTX3
Ca^{2+}	+	+	-
Phosphocholine	+	-	-
Phosphoethanolamine	+	+	-
MOβDG	-	+	-
C1q	+	+	+
Type IV collagen	ND	+	-
Fibronectin	ND	+	-

ND: test not performed

TABLE 2 *IN VIVO* ANTICANCER ACTIVITY OF PTX3

Clone[1]	Reject[2]
Parent P815 (control)	4/25
P815-AS1 (antisense)	3/8
P815-PTX3-1 (sense)	14/14*

1 : 1 x 10^5 cells of the clone indicated were injected subcutaneously.

2 : Number of animals that definitely reject the tumour out of total number of animals in which it took.

* : $p<0.01$ as compared both to mice treated with parent cells and to mice treated with cells of the antisense clones (Fisher test).

Brief description of drawings

Figure 2: PTX3 binding to C1q. Panel A shows the binding of the supernatant of the culture containing PTX3 (sense) and of the purified protein to C1q and C1s immobilised on plate. The binding is assessed as optical density (O.D.) at 405 nm. Panel B shows the saturation curve obtained with the biotinylated protein. The kinetic parameters were calculated using the non-linear fitting statistical method.

Figure 3: PTX3-induced leukocyte recruitment: 1 μg of FTX3 is injected into a subcutaneous pocket induced in the back of CD 1 mice by inoculation of 5 ml of air.

Figure 4: PTX3-induced leukocyte recruitment in normal animals and in genetically modified animals without, C1q. PTX3 is injected into a subcutaneous induced on the back of the animals.

Sequence SEQ. ID. NO 1: Amino acid sequence of human FITX3. The underlined amino acids constitute the peptide signal. Mature hPTX3 consists of 364 amino acids.

Sequence SEQ. ID. NO 2: Nucleotide sequence of fragment of cDNA of human PTX3. Upper case letters denote nucleotides coding for the protein, while lower case letters denote regions at 3' and 5'not translated but present in the construct.

CLAIMS

1. Orally, parenterally, transdermally or subcutaneously administrable pharmaceutical composition containing as active ingredient the amino acid sequence of the long pentraxin PTX3, and a pharmacologically acceptable excipient.
2. Composition according to claim 1, in which the sequence of the long pentraxin PTX3 is the sequence of naturally occurring PTX3.
3. Composition according to claim 2, in which the sequence of the long pentraxin FITX3 is the sequence of human PTX3.
4. Composition according to claims 1-3, for the treatment of infectious and inflammatory diseases or tumours.
5. Composition according to claim 4, for the treatment of diseases caused by bacteria, fungi, protozoa o viruses.
6. Method of gene therapy for the treatment of tumour conditions, comprising:

   (a) taking samples of cells from a patient suffering from a cancer;

   (b) transfecting such cells with an expression vector containing the complete cDNA sequence coding for the long pentraxin FITX3 or one of its functional derivatives; and (c) inoculating said patient with such transfected cells.

7. Method of gene therapy for the treatment of tumour conditions comprising:

a) preparation of an expression vector of viral origin containing the complete cDNA sequence coding for the long pentraxin PXT3 or one of its functional derivatives; and

b) injection of the expression vector thus obtained into a patient suffering from a cancer.

8. Method according to claim 7, in which the expression vector of viral origin is an adenovirus or retrovirus.

9. Use of the long pentraxin PTX3 for the preparation of a medicament for the treatment of infectious, inflammatory or tumour diseases.

10. Use according to claim 9, in which the long pentraxin PTX3 is the human pentraxin having sequence SEQ. ID. NO: 1.

11. Use according to claim 10, for the preparation of a medicament for the treatment of diseases caused by bacteria, fungi, protozoa or viruses.

12. Use of cDNA coding for the long pentraxin PTX3 or one of its functional derivatives, said long pentraxin PTX3 or said functional derivatives having activity in the treatment of infectious and inflammatory diseases or tumours, for the preparation of expression vectors containing such cDNA to be

used in gene therapy methods for the treatment of tumour conditions.

FIGURE 1

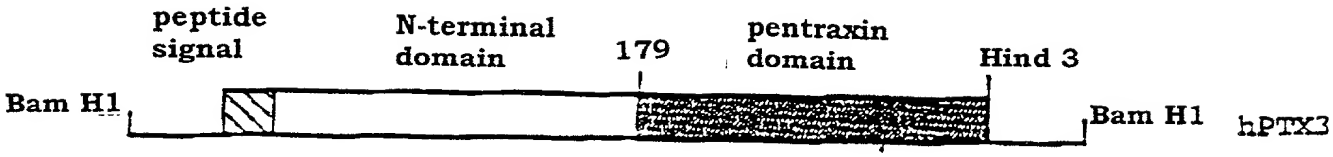


FIGURE 2

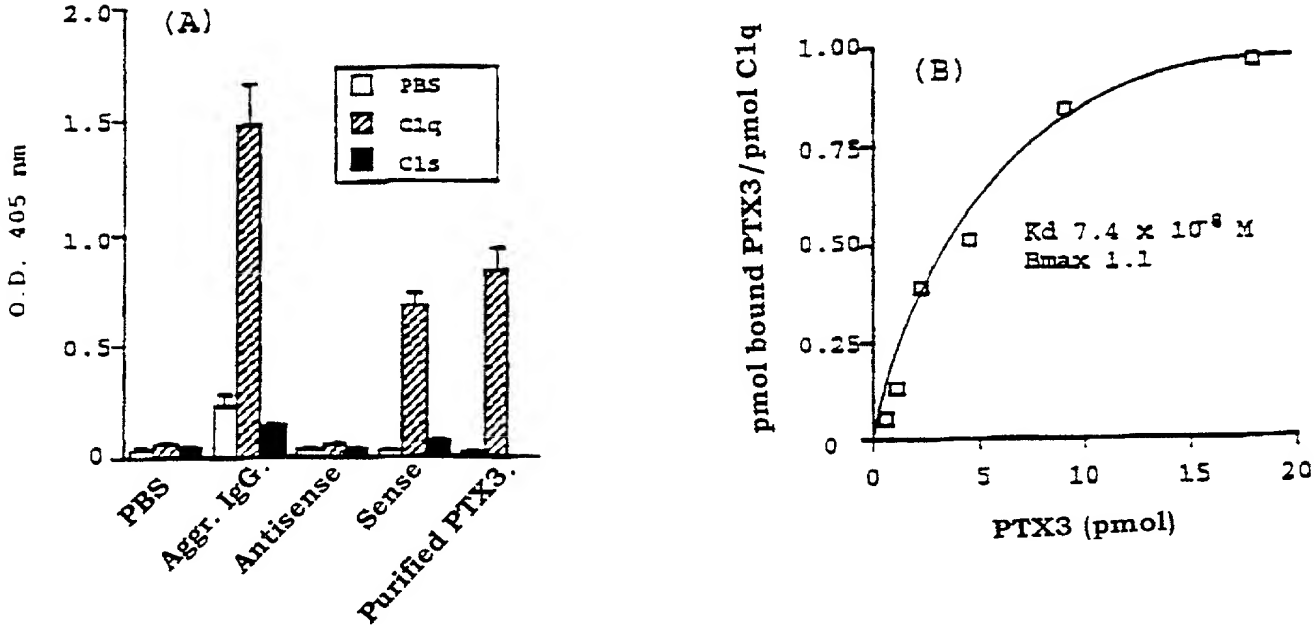

(A)
O.D. 405 nm
2.0
1.5
1.0
0.5
0
PBS
C1q
C1s
PBS
Aggr. IgG.
Antisense
Sense
Purified PTX3.
(B)
pmol bound PTX3/pmol C1q
1.00
0.75
0.50
0.25
0
Kd 7.4 x 10⁻⁸ M
Bmax 1.1
0
5
10
15
20
PTX3 (pmol)

FIGURE 3

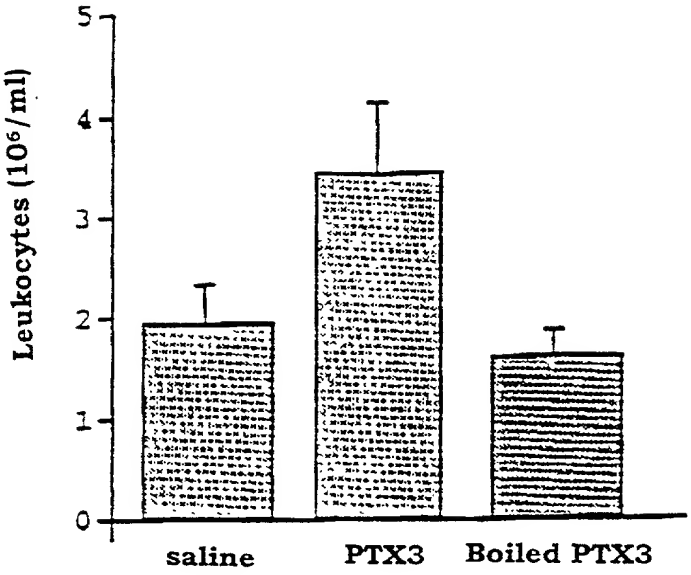
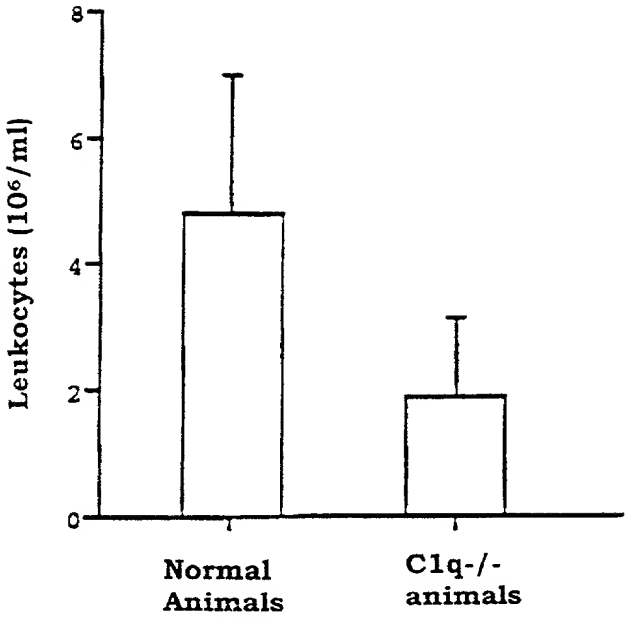


FIGURE 4



SEQ. ID. NO:
Sequence 1

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RGELQRLREE	LGRLAESLAR	PCAPGAPAEA	RLTSALDELL	121
QATRDAGRRL	ARMEGAEAQR	PEEAGRALAA	VLEELRQTRA	161
DLHAVQGWAA	RSWLPAGCET	AILFPMRSKK	IFGSVHPVRP	201
MRLESFSACI	WVKATDVLNK	TILFSYGTKR	NPYEIQLYLS	241
YQSIVFVVGG	EENKLVAEAM	VSLGRWTHLC	QTWNSEEGLT	281
SLWVNGELAA	TTVEMATGHI	VPEGGILQIG	QEKNGCCVGG	321
GFDETLAFSG	RLTGFNIWDS	VLSNEEIRET	GGAESCHIRG	361
NIVGWGVTEI	QPHGGAQYVS			381

SEQ. ID. NO:

Sequence 2

```
        ctca aacttcagctc acttgagagc ctcctcccgc cagcctgtgga aagaactttg    54
  5     cgtcctctcca gcaATGCATC TCCTTGCGAT TCTGTTTTGT GCTCTCTGGT CTGCAGTGTT   114
        GGCCGAGAAC TCGGATGATT ATGATCTGAT GTATGTGAAT TTGGACAACG AAATAGACAA   174
        TGGACTCCAT CCCACTGAGG ACCCCACGCC GTGCGACTGC GGTCAGGAGC ACTCGGAATG   234
        CGACAAGCTC TTCATCATGC TGGAGAACTC GCAGATGAGA GAGCGCATGC TGCTGCAAGC   294
        CACGGACGAC GTCCTGCGGG GCGAGCTGCA GAGGCTGCGG GAGGAGCTGG GCCGGCTCGC   354
  10    CGAAAGCCTG GCGAGCCCGT GCGCGCCGGG GGCTCCCGCA GAGGCCAGGC TGACCAGTGC   414
        TCTGGACGAG CTGCTGCAGG CGACCCGCGA CGCGGGCCGC AGGCTGGCGC GTATGGAGGG   474
        CGCGGAGGCG CAGCGCCCAG AGGAGGCGGG GCGCGCCCTG GCCGCGGTGC TAGAGGAGCT   534
        GCGGCAGACG CGAGCCGACC TGCACGCGGT GCAGGGCTGG GCTGCCCGGA GCTGGCTGCC   594
        GGCAGGTTGT GAAACAGCTA TTTATTCCC AATGCGTTCC AAGAAGATTT TTGGAAGCGT   654
  15    GCATCCAGTG AGACCAATGA GGCTTGAGTC TTTTAGTGCC TGCATTTGGG TCAAAGCCAC   714
        AGATGTATTA AACAAAACCA TCCTGTTTTC CTATGGCACA AAGAGGAATC CATATGAAAT   774
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        AGAGGAAGGG CTCACATCCT TGTGGGTAAA TGGTGAACTG GCGGCTACCA CTGTTGAGAT   954
  20    GGCCACAGGT CACATTGTTC CTGAGGGAGG AATCCTGCAG ATTGGCCAAG AAAAGAATGG  1014
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<150> RM97A000796
<151> 1997-12-19

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Case No. _____

Nixon & Vanderhye P.C. (12/97)

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: ***"Pharmaceutical compositions containing the long pentraxin PTX3"***

the specification of which (**check** applicable **box(s)**):

is attached hereto

was filed on _____ as U.S. Application Serial No. _____

X was filed as PCT International application No. **PCT/IT 98/00364** on **December 16, 1998**

and (if applicable to U.S. or PCT application) was amended on _____

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Priority Foreign Application(s):

Application Number	**Country**	**Day/Month/Year Filed**
RM97A000796	ITALY	**19.12.1997**

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number	**Date/Month/Year Filed**

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT international applications listed above or below and, insofar as the subject matter of each of the claims of this application is not disclosed in such prior applications in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. 1.56 which occurred between the filing date of the prior applications and the national or PCT international filing date of this application:

Prior U.S./PCT Application(s): Application Serial No.	**Day/Month/Year Filed**	**Status: patented pending, abandoned**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And I hereby appoint **NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8th Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed)**, and the following attorneys thereof (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Arthur R. Crawford, 25327; Larry S. Nixon, 25640; Robert A. Vanderhye, 27076; James T. Hosmer, 30184; Robert W. Faris, 31352; Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Duane M. Byers, 33363; Jeffry H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr. 29366; Thomas E. Byrne, 32205; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Alan M. Kagen, 36178; William J. Griffin, 31260; Robert A. Molan, 29834; B. J. Sadoff, 36663; James D. Berquist, 34776; Updeep S. Gill, 37334.

1.00 Inventor's Signature: X Barbara Bottazzi Date: _____

Inventor: **Barbara** (first) | MI | **BOTTAZZI** (last) | **Italian** (citizenship)

Residence: (city) **Milan** ITX (state/country) **ITALY**

Post Office Address: **28/2, Via Cascina Bianca**

(Zip Code) **I 20142**

2.00 Inventor's Signature: X Martino Introna Date: _____

Inventor: **Martino** (first) | MI | **INTRONA** (last) | **Italian** (citizenship)

Residence: (city) **Milan** ITX (state/country) **ITALY**

Post Office Address: **12/I, Via Balzarotti**

(Zip Code) **I-20017**

3.00 Inventor's Signature: X Alberto Mantovani Date: _____

Inventor: **Alberto** (first) | MI | **MANTOVANI** (last) | **Italian** (citizenship)

Residence: (city) **Milan** ITX (state/country) **ITALY**

Post Office Address: **4, Largo Brasilia**

(Zip Code) **I-20100**

FOR ADDITIONAL INVENTORS, check box ☐ and attach sheet with same information and signature and date for each.

Case No. _____

Nixon & Vanderhye P.C. (12/97)

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: ***"Pharmaceutical compositions containing the long pentraxin PTX3"***

the specification of which (**check** applicable **box(s)**):

is attached hereto

was filed on _____ as U.S. Application Serial No. _____

X was filed as PCT International application No. **PCT/IT 98/00364** on **December 16, 1998**

and (if applicable to U.S. or PCT application) was amended on _____

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Priority Foreign Application(s):

Application Number	**Country**	**Day/Month/Year Filed**
RM97A000796	ITALY	**19.12.1997**

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

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Prior U.S./PCT Application(s): Application Serial No.	**Day/Month/Year Filed**	**Status: patented pending, abandoned**

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Inventor's Signature: X Annunciata Vecchi Date: _____

Inventor: **Annunciata** | | **VECCHI** | **Italian**
(first) | MI | (last) | (citizenship)

Residence: (city) **Milan** ITX (state/country) **ITALY**

Post Office Address: **11, Via Spontini**

(Zip Code) **I-20131**

Inventor's Signature: _____ Date: _____

Inventor: _____ | _____ | _____ | _____
(first) | MI | (last) | (citizenship)

Residence: (city) _____ (state/country) _____

Post Office Address: _____

(Zip Code) _____

Inventor's Signature: _____ Date: _____

Inventor: _____ | _____ | _____ | _____
(first) | MI | (last) | (citizenship)

Residence: (city) _____ (state/country) _____

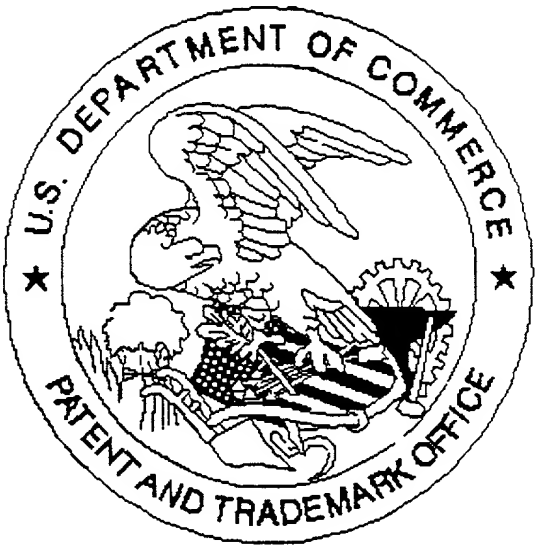
Post Office Address: _____

(Zip Code) _____

FOR ADDITIONAL INVENTORS, check box ☐ and attach sheet with same information and signature and date for each.

United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division



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☐ Page(s)_____ of _____ were not present for scanning. (Document title)

☐ Page(s)_____ of _____ were not present for scanning. (Document title)

☑ Scanned copy is best available. Drawings